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PHYLOGEOGRAPHY OF THE PANTROPICAL SEA URCHIN *EUCIDARIS* IN RELATION TO LAND BARRIERS AND OCEAN CURRENTS

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Abstract.—The pantropical sea urchin genus *Eucidaris* contains four currently recognized species, all of them allopatric: *E. metularia* in the Indo-West Pacific, *E. thouarsi* in the eastern Pacific, *E. tribuloides* in both the western and eastern Atlantic, and *E. clavata* at the central Atlantic islands of Ascension and St. Helena. We sequenced a 640-bp region of the cytochrome oxidase I (COI) gene of mitochondrial DNA to determine whether this division of the genus into species was confirmed by molecular markers, to ascertain their phylogenetic relations, and to reconstruct the history of possible dispersal and vicariance events that led to present-day patterns of species distribution. We found that *E. metularia* split first from the rest of the extant species of the genus. If COI divergence is calibrated by the emergence of the Isthmus of Panama, the estimated date of the separation of the Indo-West Pacific species is 4.7–6.4 million years ago. This date suggests that the last available route of genetic contact between the Indo-Pacific and the rest of the tropics was from west to east through the Eastern Pacific Barrier, rather than through the Tethyan Sea or around the southern tip of Africa. The second cladogenic event was the separation of eastern Pacific and Atlantic populations by the Isthmus of Panama. *Eucidaris* at the outer eastern Pacific islands (Galapagos, Isla del Coco, Clipperton Atoll) belong to a separate clade, so distinct from mainland *E. thouarsi* as to suggest that this is a different species, for which the name *E. galapagensis* is revived from the older taxonomic literature. Complete lack of shared alleles in three allozyme loci between island and mainland populations support their separate specific status. *Eucidaris galapagensis* and *E. thouarsi* are estimated from their COI divergence to have split at about the same time that *E. thouarsi* and *E. tribuloides* were being separated by the Isthmus of Panama. Even though currents could easily convey larvae between the eastern Pacific islands and the American mainland, the two species do not appear to have invaded each other's ranges. Conversely, the central Atlantic *E. clavata* at St. Helena and Ascension is genetically similar to *E. tribuloides* from the American and African coasts. Populations on these islands are either genetically connected to the coasts of the Atlantic or have been colonized by extant mitochondrial DNA lineages of *Eucidaris* within the last 200,000 years. Although it is hard to explain how larvae can cross the entire width of the Atlantic within their competent lifetimes, COI sequences of *Eucidaris* from the west coast of Africa are very similar to those of *E. tribuloides* from the Caribbean. F_{ST} statistics indicate that gene flow between *E. metularia* from the Indian Ocean and from the western and central Pacific is restricted. Low gene flow is also evident between populations of *E. clavata* from Ascension and St. Helena. Rates of intraspecific exchange of genes in *E. thouarsi, E. galapagensis*, and *E. tribuloides*, on the other hand, are high. The phylogeny of *Eucidaris* confirms Ernst Mayr's conclusions that major barriers to the dispersal of tropical echinoids have been the wide stretch of deep water between central and eastern Pacific, the cold water off the southwest coast of Africa, and the Isthmus of Panama. It also suggests that a colonization event in the eastern Pacific has led to speciation between mainland and island populations.

Key words.—Biogeography, cytochrome oxidase, gene flow, islands, mitochondrial DNA, ocean currents, sea urchins.

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In 1954 Ernst Mayr published an article on geographic speciation of tropical echinoids (Mayr 1954). In this paper, he plotted the ranges of species belonging to 16 sea urchin, sand dollar, and heart urchin genera as given in Mortensen's (1928–1951) monograph, and reached the conclusion that their geographic distributions were consistent with allopatric speciation. Geographical barriers seen by Mayr as causing speciation were the wide stretch of deep water dividing the eastern Pacific from the central Pacific (the Eastern Pacific Barrier), the Isthmus of Panama, and the cold waters off southwest Africa. He wrote that the genus *Eucidaris* "illustrates geographic speciation almost diagrammatically."

Mayr reached these conclusions while recognizing that he was dealing with morphospecies rather than biological species and that echinoid systematics were still at the stage of alpha taxonomy. We now have the tools to investigate what Avise et al. (1987) have called "phylogeography," that is, intra- and interspecific phylogenies, which in combination with distributional information can help deduce patterns and causes of divergence and speciation. Molecular phylogeography can provide information difficult to obtain from other characters, such as the existence of sibling species and the timing of splits between populations (Palumbi 1996, 1997). The present paper is an attempt to apply molecular tools to the species of the genus *Eucidaris* to evaluate Mayr's conclusions.

Eucidaris belongs to the subclass Perischoechinoidea, separated from all other extant Echinoidea at least since the Triassic (Durham 1966; Smith 1984). The fossil record of *Eucidaris* dates back to the Upper Eocene (Fell 1966; Cutress 1980), approximately 50 million years ago (mya). The genus is pantropical, with all of its species concentrated in shallow water (< 570 m; Mortensen 1928–1951). Its four recognized morphospecies have adjacent but nonoverlapping geographical distributions. *Eucidaris metularia* ranges from the east coast of Africa to the central Pacific. *Eucidaris thouarsi* is found in the eastern Pacific. *Eucidaris tribuloides* is distributed from the Atlantic coast of tropical America to the western coast of Africa. *Eucidaris clavata* is endemic to the central Atlantic islands of Ascension and St. Helena. The morpho-

logical differences between the species are slight. There are doubts in the taxonomic literature as to whether the Galapagos populations of *Eucidaris* belong to *E. thouarsi* or whether they constitute a separate species, *E. galapagensis* (Mortensen 1928–1951, vol. I, p. 399) and as to whether *E. clavata* is a separate species from *E. tribuloides* (Mortensen 1928–1951, vol. I, p. 411). *Eucidaris tribuloides* on the African coast has been recognized as a separate subspecies, *E. tribuloides africana* (Mortensen 1928–1951, vol. I, p. 406).

We used sequence data from a 640-bp fragment of the cytochrome oxidase I (COI) region of mitochondrial DNA (mtDNA) and isozymes to determine the validity of species described on the basis of morphology and to ascertain their phylogenetic relations. Specifically, we were interested in answering the following questions: (1) Is each geographic isolate a separate evolutionary lineage? (2) To what extent do the accepted morphospecies coincide with mtDNA clades? (3) What is the order and timing of cladogenic events, and what can they tell us about the effects of presumed geographic barriers on the speciation of tropical marine organisms?

Materials and Methods

Collections

One hundred twelve individuals were collected for mtDNA sequencing at the following locations for each species (see Fig. 1): *E. metularia*: Reunion, Indian Ocean (n = 4); Ishigaki-Jima, Sakishima Islands, Japan (n = 2); Sesoko, Okinawa-Jima, Ryukyu Islands, Japan (n = 4); Guam, west Pacific (n = 5); and Hawaii (n = 15, two specimens from Lanai, one from Maui, 12 from Oahu); *E. thouarsi*: Galapagos (n = 7, two specimens from each of the islands of Bartolomé, Fernandina, and Genovesa, and one from Isabela); Isla del Coco (n = 6); Clipperton Atoll (n = 1); Guaymas, Sea of Cortez, Mexico (n = 6); and Taboguilla Island, Bay of Panama (n = 4); *E. tribuloides*: Carrie Bow Cay, Belize (n = 11); Cochino Pequeño, Bay Islands, Honduras (n = 2); San Blas Islands, Panama (n = 10); Puerto Rico (n = 4); St. John, U.S. Virgin Islands (n = 3); Cayman Brac, Cayman Islands (n = 1); Tamandaré, Brazil (n = 4); Sao Tomé, Gulf of Guinea (n = 10); Ada, Ghana, Gulf of Guinea (n = 1); *E. clavata*: St. Helena (n = 7); Ascension (n = 5). We rooted the phylogenetic tree of *Eucidaris* with one individual of *Phyllacanthus imperialis* from Rottnest Island, western Australia. Cladistic analysis of morphological characters suggests that *Phyllacanthus* is the sister genus of *Eucidaris* (Smith and Wright 1989). Samples were preserved in 95% ethanol, in high-salt DMSO buffer (Seutin et al. 1991), or in liquid N_2. Forty-two individuals were collected for isozyme analysis at Isla del Coco (n = 20) and at Galapagos (n = 22, two individuals from Isabela, five from Bartolomé, four from Genovesa, six from Marchena, two from Santiago, three from Fernandina); all were frozen in liquid N_2.

Mitochondrial DNA

We amplified and sequenced 640 nucleotides from the COI region, corresponding to positions 6448 (or, depending on the primer, 6499) to 7128 of the mitochondrial genome of *Strongylocentrotus purpuratus* (Jacobs et al. 1988). Genomic DNA extractions, polymerase chain reaction (PCR) amplification, PCR product purification, and DNA sequencing were carried out as described previously (Lessios et al. 1998). Primers for both PCR and sequencing were: either COI-f 5′ (CCTGCAGGAGGAGGAGAYCC) or COI-p 5' (GGTCACCCAGAAGTGTACAT) and COI-a 5′ (AGTATAAGCGTCTGGGTAGTC). All individuals were sequenced in both directions. Phylogenies from mtDNA data were constructed with test version 4.0d64 of PAUP*, written by David L. Swofford and used with his permission, and with PUZZLE 4.0, written by Strimmer and von Haeseler (1996). Other analyses were performed with SEQUENCER 4.0, an Apple Macintosh program written by B. D. Kressing and available from him. Sequences have been deposited in GenBank under accession nos. AF063309–AF063399 and AF107700–AF107721.

Isozymes

Because the COI sequence data indicated that *Eucidaris* from the outer eastern Pacific islands (Galapagos, Isla del Coco, and Clipperton) were a distinct lineage from *E. thouarsi* from the American coast (see results), isozymes were used to determine whether genetic discontinuities in the eastern Pacific extended to the nuclear genome. Twelve loci were assayed in animals from the Galapagos and Isla del Coco in the same buffers and with the same staining recipes as those of Bermingham and Lessios (1993). The data were compared with data from the same loci obtained previously by these authors from coastal populations at Mexico and Panama. Presumptive alleles were standardized by running individuals from Panama on the same gels as those from Galapagos and Isla del Coco. The assayed loci were: acid phosphatase (*Acph*), aspartate aminotransferase (*Got-1, Got-2*), fructokinase (*Fk*), hexokinase (*Hk*), malate dehydrogenase (*Mdh-1*), L-leucyl-L-tyrosine peptidase (*Peplt-1*), phosphoglucose isomerase (*Pgi*), phosphoglucomutase (*Pgm*), octanol dehydrogenase (*Odh*), superoxide dismutatse (*To*), and xanthine dehydrogenase (*Xdh*). Calculation and comparison of jackknifed average Nei's (1978) genetic distances were performed according to the method of Mueller and Ayala (1982), employing program NEIC (Lessios 1990).

Results

Phylogenetic Groupings and Genetic Similarities

Figure 2 presents a maximum-likelihood reconstruction of the phylogeny of *Eucidaris*, as inferred from the determined COI sequences. In addition to the presented tree produced by the quartet puzzling algorithm of Strimmer and von Haeseler (1996), we also reconstructed phylogeny using the neighbor-joining algorithm of Saitou and Nei (1987), with distances corrected by Hasegawa et al.'s (1985) model of base substitution. The neighbor-joining tree was bootstrapped 1000 times. When branches with less than 70% support were collapsed, the tree topologies produced by the two techniques were identical, except for some minor rearrangements of the terminal branches.

There is strong support for the existence of four clades (see Fig. 2). The Indo-Pacific *E. metularia* (clade A) is an outgroup to all other *Eucidaris*. We were able to obtain spec-

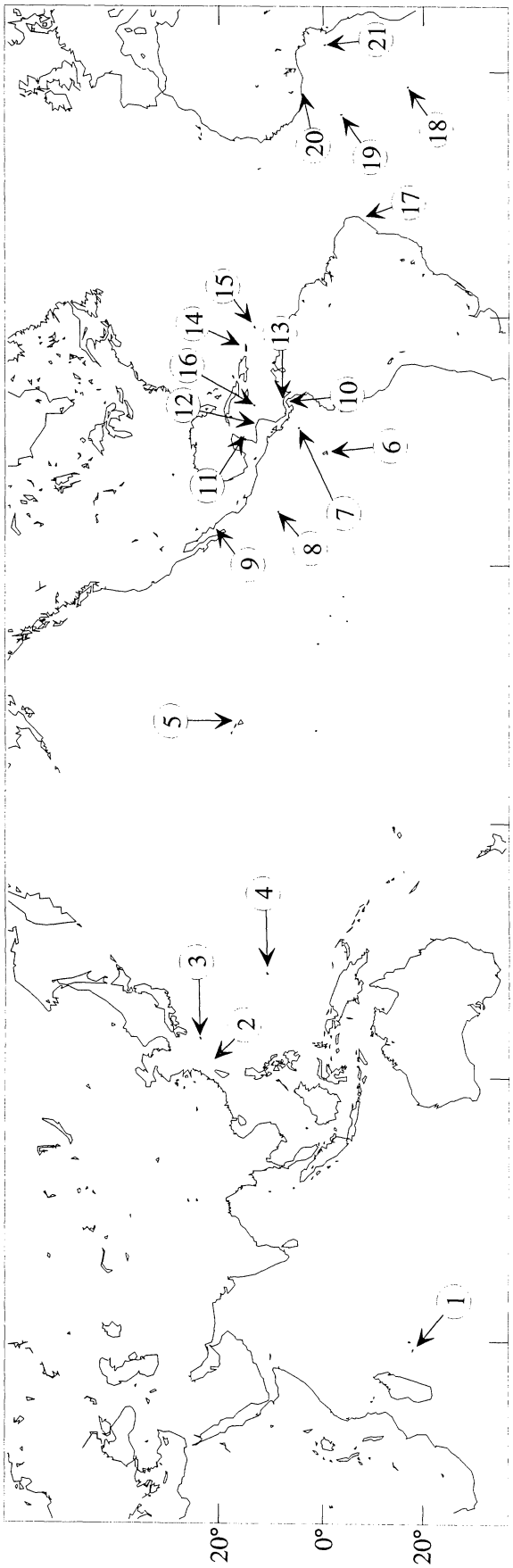

FIG. 1. Localities where specimens were collected. (1) Reunion Island; (2) Ishigaki, Ishigaki-Jima, Japan; (3) Sesoko, Okinawa-Jima, Ryukyu Islands, Japan; (4) Guam; (5) Hawaii; (6) Galapagos; (7) Isla del Coco; (8) Clipperton Atoll; (9) Guaymas, Mexico; (10) Taboguilla, Bay of Panama; (11) Carrie Bow Cay, Belize; (12) Cochino Pequeño, Honduras; (13) San Blas, Panama; (14) Puerto Rico; (15) St. John, U.S. Virgin Islands; (16) Cayman Brac, Cayman Islands; (17) Tamandaré, Brazil; (18) St. Helena; (19) Ascension; (20) Ghana; (21) Sao Tomé.

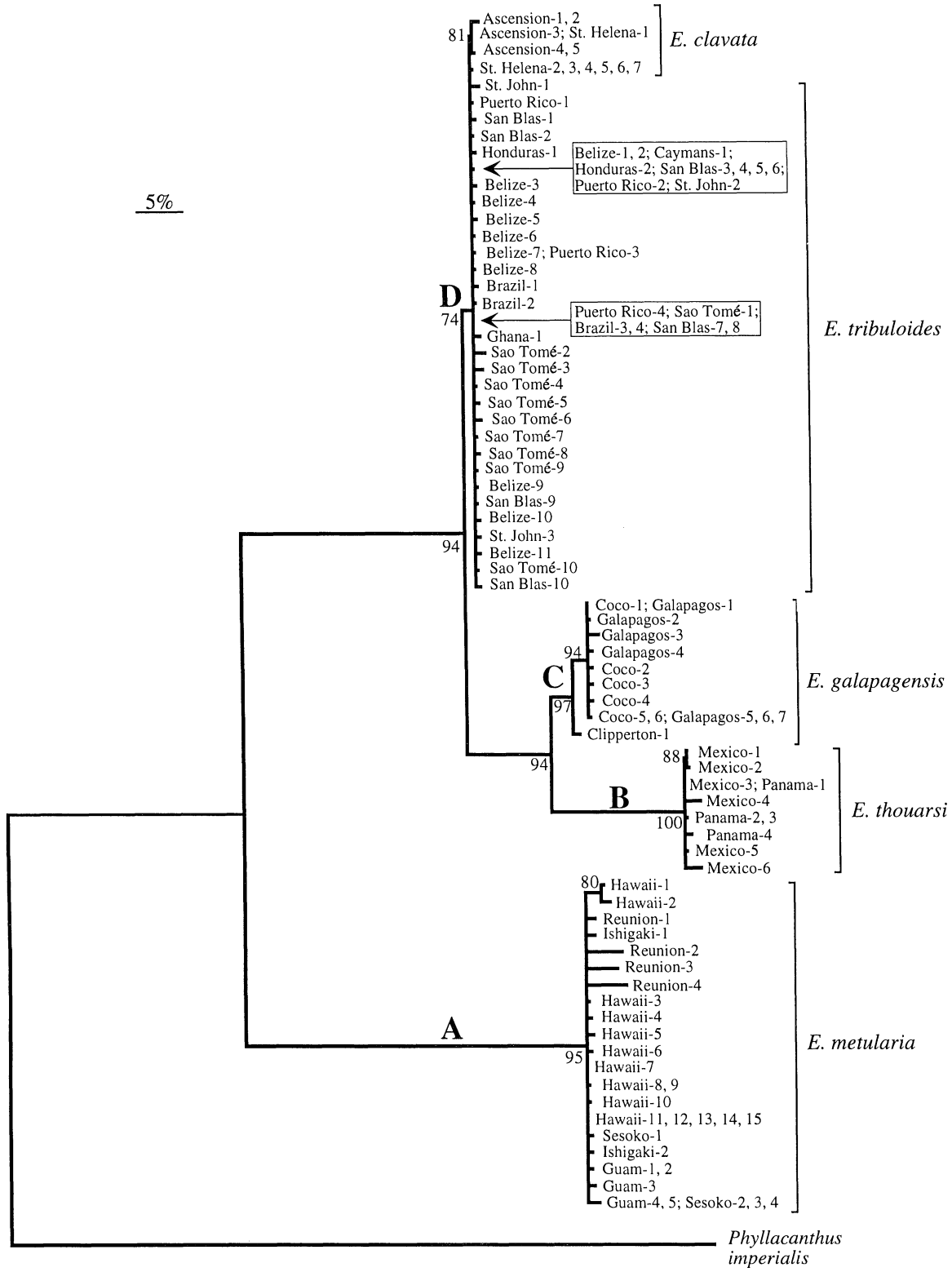

FIG. 2. Maximum-likelihood tree of COI haplotypes of *Eucidaris*, generated by the quartet puzzling technique of Strimmer and von Haeseler (1996), using Hasegawa et al.'s (1985) model of base substitution. Multiple labels next to a branch indicate that the same haplotype was observed in more than one individual. Numbers next to nodes indicate percent of quartets that support them. Only nodes with more than 70% support are shown. Letters next to branches mark major clades.

TABLE 1. Number of sampled individuals and gene frequencies in 12 electrophoretically assayed loci of eastern Pacific populations of *Eucidaris*. Locus abbreviations are explained in the text. Allele designations indicate relative distance from the origin. Data for *E. thouarsi* are from Bermingham and Lessios (1993). *Got-2, Odh,* and *Xdh* were monomorphic in all populations.

		E. thouarsi		*E. galapagensis*	
Locus	Allele	Mexico	Taboguilla	Isla del Coco	Galapagos
Acph	*n*	30	28	18	22
	90	0.133	0.196	0.472	0.546
	100	0.833	0.786	0.528	0.455
	110	0.033	0.018	0.000	0.000
Fk	*n*	19	33	20	22
	80	0.000	0.015	0.000	0.000
	90	1.000	0.924	0.275	0.273
	98	0.000	0.000	0.650	0.568
	100	0.000	0.061	0.000	0.000
	120	0.000	0.000	0.075	0.159
Got-1	*n*	32	76	16	22
	100	1.000	1.000	0.000	0.000
	110	0.000	0.000	1.000	1.000
Hk	*n*	29	44	20	22
	90	0.000	0.045	0.000	0.000
	100	1.000	0.955	0.000	0.000
	110	0.000	0.000	1.000	1.000
Mdh-1	*n*	37	55	19	22
	97	0.000	0.000	0.842	0.932
	98	0.000	0.009	0.000	0.000
	100	0.405	0.109	0.158	0.068
	103	0.595	0.855	0.000	0.000
	105	0.000	0.027	0.000	0.000
Peplt-1	*n*	33	56	20	22
	80	0.879	0.000	0.000	0.000
	90	0.121	0.170	0.100	0.114
	100	0.000	0.750	0.900	0.886
	110	0.000	0.080	0.000	0.000
Pgi	*n*	30	26	19	—
	94	0.000	0.077	0.000	—
	95	0.000	0.077	0.105	—
	97	0.050	0.000	0.000	—
	100	0.900	0.692	0.711	—
	103	0.050	0.154	0.184	—
Pgm	*n*	25	35	18	22
	70	0.020	0.057	0.000	0.000
	90	0.100	0.157	0.028	0.023
	100	0.640	0.671	0.944	0.977
	110	0.200	0.071	0.028	0.000
	120	0.040	0.043	0.000	0.000
To	*n*	22	46	20	22
	100	0.000	0.000	1.000	1.000
	105	1.000	1.000	0.000	0.000

imens of this species only from five localities, and with this limited sample it is difficult to draw conclusions regarding the number of distinct lineages that are truly represented in this vast oceanic expanse. However, despite the long distance separating Reunion from Guam (a straight-line distance of > 10,000 km) and from Hawaii (17,000 km), the mtDNA lineages do not form separate clades, indicating that even very distant populations of *E. metularia* have not evolved independently.

The data clearly indicate that there are two distinct lineages of *Eucidaris* in the eastern Pacific. All coastal specimens from Mexico and Panama belong to one clade (B), which represents *E. thouarsi sensu stricto*. Specimens from Galapagos, Isla del Coco and the Clipperton Atoll cluster in a separate clade (C), which is likely to be a different species. Reviving Döderlein's (1887) name, we have labeled it as *E. galapagensis*. The 640 mtDNA bases we sampled contain 33 nucleotide sites in which *E. thouarsi* and *E. galapagensis* are consistently different. In keeping with the high degree of conservatism of amino acid composition of COI, all of these fixed differences are silent. The single specimen we were able to obtain from Clipperton Atoll is sufficiently different from all others coming from eastern Pacific islands (Kimura [1980] two-parameter corrected percent difference [K_2] of 1.79%) to suggest that it may belong to an additional evolutionary lineage. The differentiation of *Eucidaris* at this remote Atoll from other populations of the eastern Pacific needs to be investigated further.

TABLE 2. Average jackknifed means of Nei's *D* distances between eastern Pacific populations, assayed for 12 electrophoretically determined loci.

	Mexico	Taboguilla	Isla del Coco
Taboguilla	0.084		
Isla del Coco	0.639	0.580	
Galapagos	0.718	0.625	0.000

Isozyme results from eastern Pacific populations confirm that *Eucidaris* populations from Isla del Coco and from the Galapagos are very similar to each other and that no gene flow has occurred between island and mainland populations during recent evolutionary time. The most common allele in five of the 12 assayed loci is different between the islands and the American coast. In three of these loci there is complete partitioning of alleles (Table 1). Nei's *D* between populations at Galapagos and Isla del Coco is less than 0.001 (Table 2), whereas the average jackknifed Nei's *D* between *E. galapagensis* and *E. thouarsi* is 0.641, which is not significantly different ($P > 0.05$) from the value of Nei's *D* (0.758) calculated from the same 12 loci (of 25 sampled by Bermingham and Lessios 1993) between *E. thouarsi* and the Caribbean *E. tribuloides*.

Clade D in Figure 2 is composed of all individuals from the Atlantic, irrespective of whether they belong to the west Atlantic *E. tribuloides*, the African *E. tribuloides africana*, or the central Atlantic *E. clavata*. Within this clade, there are no resolved subclades, except for one joining all haplotypes from Ascension and one from St. Helena (which is identical to one of the Ascension haplotypes). The low degree of phylogenetic structure is the result of high haplotype similarity. There are no fixed differences between Caribbean, Brazilian, and African *E. tribuloides*. A single transition at a third codon position is diagnostic between *E. clavata* from Ascension (and one individual from St. Helena) and *E. tribuloides* from the American and African coasts. A third position transition at a different site distinguishes the remaining six individuals from St. Helena from continental *E. tribuloides*. Thus, some differentiation exists between *E. clavata* and *E. tribuloides*. However, the mtDNA divergence between *Eucidaris* from the Caribbean and the central Atlantic islands (average K_2 = 0.49) is no larger than the differentiation seen between *E. clavata* from St. Helena and from Ascension (K_2 = 0.64).

TABLE 3. F_{ST}-values (below the diagonal) and estimated number of female propagules (N_em; above the diagonal) between populations of *Eucidaris* in which at least four individuals were sampled. Calculations follow Hudson et al. (1992); ud, undefined value of N_em because F_{ST} for this comparison is negative.

Indo-West Pacific

Species	Region	Reunion	Ryukyu	Guam	Hawaii
E. metularia	Reunion	—	0.79	0.53	0.22
E. metularia	Ryukyu	0.39	—	ud	0.88
E. metularia	Guam	0.48	−0.09	—	1.23
E. metularia	Hawaii	0.70	0.36	0.29	—

Eastern Pacific

Species	Region	Panama	Mexico	Isla del Coco	Galapagos
E. thouarsi	Panama	—	ud	0.02	0.02
E. thouarsi	Mexico	−0.03	—	0.04	0.04
E. galapagensis	Isla del Coco	0.96	0.93	—	ud
E. galapagensis	Galapagos	0.95	0.93	−0.06	—

Atlantic

Species	Region	Ascension	St. Helena	Puerto Rico	Belize	San Blas	Brazil	Sao Tomé
E. clavata	Ascension	—	0.19	0.51	1.44	0.66	0.55	1.11
E. clavata	St. Helena	0.73	—	0.53	6.28	1.12	0.52	4.77
E. tribuloides	Puerto Rico	0.50	0.49	—	ud	ud	ud	ud
E. tribuloides	Belize	0.26	0.07	−0.36	—	ud	ud	4.50
E. tribuloides	San Blas	0.43	0.31	−0.10	−0.07	—	596.41	2.31
E. tribuloides	Brazil	0.48	0.49	−0.08	−0.22	0.01	—	ud
E. tribuloides	Sao Tomé	0.31	0.09	−0.16	0.10	0.18	−0.11	—

Similarly, the difference between *E. clavata* and *E. tribuloides africana* (K_2 = 0.75) is only slightly larger than the differences within *E. clavata*.

Estimates of gene flow within the Indo-West Pacific based on F_{ST}-values (Table 3) suggest that, despite the lack of distinct evolutionary lineages, some degree of genetic isolation exists between the Indian and the Pacific Oceans. Although populations from Guam and the Ryukyu Islands are panmictic, and Hawaii and Guam are also connected by fairly high gene flow, Reunion, as might be expected, is more differentiated. Gene flow between this Indian Ocean island and the western Pacific is less than the equivalent of one female propagule per generation. Overall, the values of F_{ST} in *E. metularia* are correlated with the straight-line distance between localities. In the eastern Pacific, mtDNA differentiation between localities within each presumed species is less than diversity within each locality. However, estimated migration between mainland *E. thouarsi* and island *E. galapagensis* is almost equal to zero, confirming our conclusion that island and mainland populations must have remained isolated for a long time.

In the Atlantic, most populations are genetically connected. The two populations in the Caribbean from which we sampled 10 or more individuals, San Blas and Belize, contain haplotypes that are more similar between than within localities, leading to a negative F_{ST}-value. *Eucidaris tribuloides* from Brazil, despite substantial distance (4000 km along the coast from the closest point in the Caribbean) and possible barriers to echinoid dispersal due to the freshwater discharge of the Orinoco and the Amazon, is also connected to the Caribbean with high levels of gene flow. Indeed, the F_{ST}-values suggest that *Eucidaris* from the entire western Atlantic belong to a single panmictic unit. The overall F_{ST}-value for comparisons among all Caribbean localities plus Brazil (−0.143) is not only negative, but also not significantly different (P = 0.938) from that generated from 500 random reshufflings of haplotypes. There is also high apparent gene flow between the American and the African coast. Each western Atlantic population of *E. tribuloides* exchanges genes with Sao Tomé at rates higher than 2.3 females per generation (Table 3). However, the genetic connections between *E. tribuloides* and *E. clavata* seem more variable. High gene flow is estimated between the African coast and the central Atlantic islands and between the central Atlantic islands and Belize. Puerto Rico, San Blas, and Brazil, despite being genetically connected with Belize and with the African coast, appear to be contributing fewer genes toward connections with *E. clavata*. Interestingly, although Ascension and St. Helena are supposed to be both inhabited by *E. clavata*, and even though the distance between them (1300 km) is smaller than that between the American and the African coast (a minimum of about 2800 km), the estimated number of female propagules exchanged between the two islands is the lowest for any comparison within the Atlantic.

Timing of Cladogenic Events

How long ago did these clades diverge from each other? Mitochondrial DNA differentiation (see Table 4), under the assumption of constant rates of evolution, can provide an estimate. This estimate cannot be very precise, because in *Eucidaris* evolution of the sequenced segment is not all that constant. If there were no rate variation between lineages, *E. metularia* would have been equidistant from all other species and *E. tribuloides* would be equidistant from *E. galapagensis* and *E. thouarsi*. However, the average K_2 distance between *E. metularia* and each of the other species ranges from 16.01% (*E. metularia-E. clavata*) to 19.85% (*E. metularia-E. gala-*

TABLE 4. Means of nucleotide percent difference between species of *Eucidaris*. Values below the diagonal are percent difference in all sites (K_2), corrected with Kimura's (1980) two-parameter model. Values on the diagonal (in bold) are average K_2-values between individuals within a species. Values above the diagonal are percent nucleotide differences in silent sites (K_s), estimated from the equations of Pamilo and Bianchi (1993) and Li (1993).

Species	Region	*E. clavata*	*E. metularia*	*E. galapagensis*	*E. thouarsi*	*E. tribuloides*
E. clavata	Central Atlantic	**0.42**	72.72	28.42	39.30	1.53
E. metularia	Indo-West Pacific	16.01	**1.12**	131.74	90.52	74.55
E. galapagensis	Central eastern Pacific	8.29	19.85	**0.58**	31.12	29.21
E. thouarsi	East eastern Pacific	10.83	18.78	9.11	**0.72**	40.28
E. tribuloides	Caribbean and African coast	0.54	16.34	8.49	11.05	**0.52**

pagensis), and the average difference of *E. tribuloides* from *E. thouarsi* (11.05 %) is 1.3 times higher than the difference of *E. tribuloides* from *E. galapagensis* (8.49%). Thus, K_2-values that would have been equal under a strict molecular clock hypothesis can vary by up to 30%. Substitution at silent sites is supposed to be more regular because of the absence of selective constraints, yet K_s-values show variation that is even higher, probably because they are based on a fraction of all sites, and thus have larger sampling error (Table 4). Thus, the cladogenic events in *Eucidaris* can only be dated within broad time constraints. If we assume that the separation of *E. tribuloides* from *E. thouarsi* and *E. galapagensis* occurred at the time of the final closure of the Isthmus of Panama 3.1 million years ago (Coates and Obando 1996) and equate this length of time with the average divergence between all east Pacific and all Atlantic sequences (K_2 = 9.52%), we obtain a calibration of 3.1% sequence divergence per million years. By this calibration, and allowing for a range of rate variation 15% on either side of the mean, *E. metularia* diverged from all other *Eucidaris* in the late Miocene/early Pliocene between 4.7 and 6.4 million years ago (average K_2 = 17.19%), and *E. galapagensis* separated from *E. thouarsi* in the Pliocene approximately 2.5–3.4 million years ago. If we were to assume that *E. clavata* no longer exchanges genes with *E. tribuloides*, the estimated date of separation would be 148,000–200,000 years ago.

DISCUSSION

Correspondence between mtDNA Clades and Biological Species

What new insights into the phylogeny of *Eucidaris* do the molecular data provide, and what general patterns of ocean biogeography can they address? The first point genetic data can establish, a point difficult to examine with previously available alpha taxonomic studies of the genus, is to demonstrate which populations exchange genes and which do not. In its extreme form of complete cessation of gene flow, this is tantamount to the demonstration of specific status.

As mentioned in the introduction, there has been disagreement as to whether the eastern Pacific harbors one or two species of *Eucidaris*. Although the name *E. thouarsi* is unquestionably applied by all authors to the populations on the western American coast, Döderlein (1887) described the form found in the Galapagos as a separate species, *E. galapagensis*. However, Clark (1925) stated that he would hesitate to recognize it even as a "valid variety." Mortensen (1928–1951) did accept *E. galapagensis* as a variety of *E. thouarsi*, but doubted that it deserved specific rank. More recent biogeographic (Maluf 1988, 1991) and ecological (Glynn et al. 1979; Glynn 1994; Wellington 1997) works have all dealt with Galapagos populations of *Eucidaris* as if they belonged to *E. thouarsi*. However, both our mtDNA and our isozyme data provide strong evidence that *E. galapagensis* is a species separate from *E. thouarsi*, from which it diverged at about the same time that the eastern Pacific was being separated from the Caribbean by the Central American isthmus. Moreover, this species is not a Galapagos endemic, but is also found at Isla del Coco and perhaps at Clipperton Atoll.

An interesting question is whether *E. galapagensis* and *E. thouarsi* are truly allopatric. Given their morphological similarity, it is possible that colonization of the mainland by *E. galapagensis* or of the islands by *E. thouarsi* could have gone undetected in faunal lists of either region. Indeed, based on morphology, we have erroneously reported *E. thouarsi* as present at Isla del Coco and the Clipperton Atoll (see table 1 in Lessios et al. 1996). Although the question cannot be answered with certainty until the western American coast is more extensively surveyed, our collections would be unlikely to have missed genotypes of the "wrong" species in localities that were sampled. Our combined mtDNA and isozyme samples come to 45 individuals from the islands (some individuals were assayed for both kinds of molecules) and 117 from the American mainland. Fifty-two additional *E. thouarsi* from the Bay of Panama and 51 from the Gulf of Chiriqui were sampled in previous isozyme studies (Lessios 1979, 1981) that included four of the loci diagnostic between *E. thouarsi* and *E. galapagensis*. These studies would have noted the presence of *E. galapagensis* genotypes in the mainland, yet none of them showed evidence of invasion of one lineage into the geographical range of the other. That no island genotypes appear to be present on the American coast is not easy to explain, given the relatively small distances involved (Isla del Coco is 500 km west of Costa Rica) and the prevailing current flow from west to east (Wyrtki 1965, 1966, 1967; Abbott 1966; Tsuchiya 1974), but neither is it all that unusual. There are 17 echinoderm species endemic to the Galapagos and nine endemic to Isla del Coco (Maluf 1991). The apparent absence of the reverse colonization, by *E. thouarsi* from the coast to the islands, is more peculiar. All other species of sea urchins found commonly on the mainland are also known from the outer islands. In at least one case, that of *Diadema mexicanum*, sequencing of mtDNA has shown that this is not another case of an undetected sibling species (Lessios et al. 1996).

Despite the geographic analogies between the eastern Pa-

cific islands of Galapagos, Coco, and Clipperton and the central Atlantic islands of St. Helena and Ascension, the case of *E. clavata* is opposite to that of *E. galapagensis*. As they have for *E. galapagensis*, echinoderm taxonomists have expressed doubts as to whether *E. clavata* should be treated as a species distinct from *E. tribuloides*. Mortensen (1928–1951, vol. I, p. 411) considered whether *E. clavata* should be just a variety of *E. tribuloides*, but decided to describe it as a separate species and continued to regard it as such in other publications (Mortensen 1932, 1933). However, he stated that *E. tribuloides* is present at Ascension along with *E. clavata* (Mortensen 1936). Pawson (1978) carried out measurements of specimens of *Eucidaris* from Ascension, St. Helena, West Africa, and the West Indies, and, on the basis of morphological differences, he concluded that *E. clavata* is a separate species to which all *Eucidaris* from both Ascension and St. Helena belong. In contrast to the case of *E. galapagensis*, however, the mtDNA similarities indicate that *E. clavata* is more likely to be a peripheral deme of *E. tribuloides*.

Although the spatial distances are formidable (Ascension to Brazil, 2300 km, Ascension to St. Helena, 1300 km, St. Helena to Africa, 1900 km), transport of larvae from the continents to the central Atlantic is not beyond the realm of possibility. The larvae of *E. thouarsi* (Emlet 1988) and (possibly) *E. tribuloides* (McPherson 1968) settle in the laboratory 25–30 days after fertilization. The southernmost limit of the range of *E. tribuloides* extends to Rio de Janeiro (Bernasconi 1955) and the easternmost limit to the Gulf of Guinea (Chesher 1966). The close genetic similarity between *E. tribuloides* from the Caribbean Sea and Brazil to the Gulf of Guinea indicates that there has been recent gene flow between these areas, despite the fact that the transit time across the tropical Atlantic, estimated from the average velocity of the equatorial undercurrent (Metcalf et al. 1962), is 43–70 days (Chesher 1966). The time estimated by Manning and Chace (1990) for larval transit from the coast of Brazil to Ascension on the equatorial counter-current is 48 days. Travel in the opposite direction, from the central Atlantic to the coast of Brazil on the westward-flowing south equatorial current (Longhurst 1962) is also a possibility. Edwards and Lubbock (1983) report *E. clavata* from St. Paul's Rocks, which is 2000 km northwest of Ascension, and 960 km northeast of the Brazilian coast, so there may be a stepping stone along the way. That F_{ST}-values suggest little genetic exchange between the two central Atlantic islands is partly due to the low mtDNA variability within each locality (see below), which tends to drive F_{ST} values upward (Charlesworth 1998). However, as pointed out, mtDNA sequence divergence between haplotypes from the two islands is larger than differentiation between central and western Atlantic populations of *Eucidaris*. As the single shared haplotype indicates, migration between St. Helena and Ascension does occasionally happen. However, each island represents a minuscule source and a minuscule target for waterborne larvae, so it is not surprising that such events are infrequent.

The alternative explanation to ongoing gene flow between the central Atlantic islands and the continental margins is that single, improbable events of colonization established the ancestors of the extant mtDNA haplotypes at each island and there has been little or no subsequent genetic exchange. St. Helena is thought to be 13.3–15.3 million years old, but Ascension has only emerged in the last 1.5 million years (Baker 1970; Mitchell-Thomé 1982). If we assume that there has not been repeated genetic contact, our calibration of the *Eucidaris* COI clock would estimate the colonization of these islands as having occurred less than 200,000 years ago. Such a recent date eliminates the possibility that colonization by extant mtDNA clades in the central Atlantic took place at a time before sea-floor spreading had established the Atlantic in its present-day dimensions (Berggren and Hollister 1974; Scheltema 1995).

TABLE 5. Sample size and average within-population percent nucleotide difference (K_2) of COI in all populations of *Eucidaris* in which more than two individuals were sampled.

Locality	*n*	K_2
Reunion	4	2.33
Ryukyu	4	0.25
Guam	5	0.31
Hawaii	15	0.63
Galapagos	7	0.44
Isla del Coco	6	0.33
Bay of Panama	4	0.36
Mexico	6	0.89
Belize	11	0.54
San Blas	10	0.31
St. John	3	0.85
Puerto Rico	4	0.28
Brazil	4	0.34
Sao Tomé	10	0.72
St. Helena	7	0.14
Ascension	5	0.25

Haplotype diversity of *Eucidaris* in the central Atlantic islands suggests that a single recent introduction is a possibility. Palumbi et al. (1997) found that *Echinometra mathaei* in Hawaii shows 0% sequence heterogeneity in COI. They explained this lack of diversity as the result of a single recent colonization event. *Eucidaris* at St. Helena has less haplotype diversity than any other population of this genus for which we sequenced three or more specimens (see Table 5). Six haplotypes are identical, while the seventh is similar to the ones from Ascension (see Fig. 2). Diversity values at Ascension are higher, but still among the lowest ones observed. Thus, although *Eucidaris* in the central Atlantic islands is not as genetically depauperate as *E. mathaei* at Hawaii and its haplotypes are closely related to those found at continental margins, it may be to a considerable extent cut off from the mainland.

Possible Causes of Isolation

Although Mayr (1954) was more inclined to think in terms of dispersal, the allopatric species of *Eucidaris*, with adjacent ranges spanning in their aggregate the entire tropics, have always been consistent with the hypothesis of a circumglobal original common stock, which became fractionated by the closure of seaways and the formation of deep oceanic stretches. The phylogenetic information added by our study cannot distinguish between biogeographic models of dispersal and vicariance, but it can provide estimates of the approximate age of separation between geographical isolates. This, in turn,

allows informed guesses about the nature of the possible barriers that resulted in the species as we see them today.

The first vicariance event in the history of extant *Eucidaris* was the isolation of the Indo-Pacific species from all others. In this regard, the phylogeny of *Eucidaris* resembles that of *Echinometra*, which also shows a deep split between species from the western Pacific and those from the eastern Pacific and the Atlantic (Palumbi 1996). Cutress (1980, p. 165) suggested that affinities between Atlantic and Indo-Pacific cidaroids can be best explained as the result of west-to-east dispersal through the Tethyan Sea. Miocene fossils of *Eucidaris* from the Azores and the Canary Islands (areas in which the genus is now extinct) support the view of uninterrupted distribution from the Atlantic to the Indian Ocean. However, our estimated time of separation of *E. metularia* suggests that circumglobal genetic connections in *Eucidaris* continued after the closure of the Tethyan Sea. Although there is considerable disagreement regarding the time of the final closure of the Tethys (Adams 1967; McKenzie 1967; Ruggieri 1967; Luyendyk et al. 1972; Berggren and Hollister 1974; Rögl and Steininger 1984; Piccoli et al. 1987; Robba 1987; Rosen and Smith 1988; Por 1989; Hallam 1994, p. 178), we are aware of no estimate that is more recent than 12 million years ago; the most widely accepted date is the early Miocene, approximately 20 million years ago (Rosen 1984; Winterbottom and McLennan 1993). Even with broad allowances for rate variation, our oldest estimate from mtDNA for the separation of *E. metularia* is 6.5 million years ago, in the late Miocene. Thus, Atlantic and eastern Pacific *Eucidaris* were still exchanging genes with the Indo-Pacific after the shallow epicontinental Tethyan Sea ceased to exist. In principle, connections were still possible in the late Miocene and early Pliocene around the southern tip of Africa, because the Benguela cold water upwelling system off southwestern Africa, which appeared in the Miocene (Diester-Haass and Schrader 1979; Siesser 1980), was not established as a continuous phenomenon until the late Pliocene (Shannon 1985). However, unlike other Indo-Pacific shallow-water echinoids, the ranges of which extend past Durban, *E. metularia* only reaches Mozambique (Clark 1925; Clark and Courtman-Stock 1976). This suggests that *Eucidaris* may be a more ''tropical'' genus, the larvae of which are less likely to cross an area of even weak upwelling. A more probable last obstacle to migration is the Eastern Pacific Barrier, the 5000-km stretch of deep water separating the central from the eastern Pacific (Ekman 1953; Briggs 1974; Vermeij 1978, p. 253, 1987; Grigg and Hey 1992). Although the Eastern Pacific Barrier has possibly existed for the entire Cenozoic (Grigg and Hey 1992), in sea urchins, as in many other groups, it is functioning to the present day as a haphazard filter, which is subject to sporadic breaches by larvae (Lessios et al. 1996), sometimes resulting in massive gene flow (Lessios et al. 1998). The presence in the eastern Pacific of mitochondrial haplotypes that belong to central Pacific species has been documented in other sea urchin genera, such as *Echinothrix* (Lessios et al. 1998), *Diadema* (Lessios et al. 1996), and *Echinometra* (Palumbi 1997). However, that central and eastern Pacific populations of some of these genera have speciated in the first place indicates that there have also been periods during which they did not maintain genetic contact. Apparently the last time that east and west Pacific *Eucidaris* exchanged genes was 4.7–6.4 million years ago.

The cause of the next cladogenic event in the history of extant species of *Eucidaris* is no mystery. The Isthmus of Panama separated *E. tribuloides* from eastern Pacific stock. We cannot be certain whether this separation of *Eucidaris* was contemporaneous with the final closing of the isthmus or whether it occurred at some point during its gradual shoaling (Coates and Obando 1996) because the transisthmian COI divergence reported here (9.5% sequence divergence when distances between *E. tribuloides* and the two east Pacific species are averaged) is similar to that found between analogously separated Atlantic and Pacific species of *Echinometra* (9.8%), but twice as large as that of species of *Diadema* (4.7%; Lessios 1998). Although it is unclear whether the lower values of transisthmian divergence in *Diadema* have resulted from slower rate of COI evolution in this genus, breaching of the isthmus at times of high sea level stands (Cronin and Dowsett 1996), continuing gene flow through mangrove swamps at the time of isthmus completion (Keller et al. 1989), or circumglobal gene flow around the southern tip of Africa, the discrepancy points to the possibility that separation of eastern Pacific and western Atlantic *Eucidaris* may be somewhat older than 3.1 million years ago. If so, the dates of other cladogenic events, dated by a calibration based on this split, may also be older, but not by so much as to alter conclusions as to the possible causes of separation. One would need to postulate a calibration of COI evolution three times slower than our estimate to accept the closure of the Tethys as a credible cause of speciation of *E. metularia*.

The final definite split in *Eucidaris* occurred in the eastern Pacific between mainland and island populations. There is no obvious extrinsic barrier to gene flow that could have caused this split. Presumably, the shoaling of the Isthmus of Panama affected ocean circulation (Maier-Reimer et al. 1990; Haug and Tiedemann 1998), but it is not clear how it did so in the eastern Pacific or how long before the final closure the shoaling altered surface currents. It appears that, like many other marine species that are endemic to the Galapagos (Walker 1966; Glynn and Wellington 1983; James 1984; Garth 1991; Kay 1991; Maluf 1991; Allen and Robertson 1994) and/or to other eastern Pacific islands (Hertlein 1963; Garth 1991; Maluf 1991; Allen and Robertson 1994; Robertson and Allen 1996), *E. galapagensis* may have speciated because larvae reached the islands and then, for unknown reasons, were cut off from the mainland. That the age of the oldest present-day Galapagos islands (see Glynn and Wellington 1983, p. 162; Chavez and Brusca 1991 and references therein) roughly agrees with the three million years ago timing of the split between *E. thouarsi* and *E. galapagensis* is probably a coincidence. The central eastern Pacific contains several ''drowned'' islands, dating back to nine million years ago (Christie et al. 1992). Land vertebrates on the Galapagos appear to have been separated from their mainland ancestors for much longer than three million years (Wyles and Sarich 1983; Rassmann 1997). Thus, the time available for peripatric speciation of *E. galapagensis* is longer than the age of any extant island.

Conclusions

Because of the lack of geographic overlap between the species, the genus *Eucidaris* represents the simplest possible case for reconstructing phylogeographic patterns in pantropical shallow water tropical genera with moderately long-lived larvae. Most of what we know about marine biogeography and barriers to dispersal for marine organisms has been based on the traditional approach of studying the number of species that are found in common in different geographic regions (e.g., Ekman 1953; Briggs 1974; Vermeij 1978, 1987). Several features of the *Eucidaris* phylogeography presented here have significance that may extend beyond this particular genus. Despite the tremendous distances involved, populations from the east and west Atlantic coasts are connected by recent gene flow, as one would have surmised from their assignment by traditional taxonomists into the same species. The central Atlantic islands of Ascension and St. Helena show only a small degree of genetic isolation from the continental margins. Estimates of the isolation of these islands, based on the percentage of endemism (e.g., Mortensen 1933; Briggs 1974; Rosewater 1975; Pawson 1978; Lubbock 1980; Edwards 1990; Manning and Chace 1990; Biernbaum 1996), may in some cases be biased upward because of the erroneous elevation of their populations to separate specific status. The offshore islands of the eastern Pacific have provided opportunities for speciation dating back to the Pliocene. Despite the existence of oceanographic conditions favoring dispersal between them and the American mainland, the island and the mainland species have not invaded each other's ranges. This isolation is not limited to the Galapagos, as suggested by traditional biogeography (Ekman 1953, p. 45; Briggs 1974, pp. 43,50), but also extends to Isla del Coco, which has been included by some biogeographers (e.g., Hertlein 1963; Briggs 1974, p. 53) in the Panamic Province. As has been suggested numerous times on the basis of many groups of animals (for review, see Lessios 1998), the Isthmus of Panama represents a distinct biogeographic barrier that closed a primary avenue of dispersal between the tropical Atlantic and tropical Pacific. The last available route of genetic contact between the Indo-Pacific and the rest of the tropics was probably from west to east through the Eastern Pacific Barrier, rather than through the Tethys or around the southern tip of Africa.

As more genera of marine organisms are examined in the same manner as we have done for *Eucidaris*, there will undoubtedly be many that do not conform to one or more of these patterns. There may be some that conform to none of them, not even those that have once been thought so obvious as to need no study, such as the assumption that there has been no migration between the Indian Ocean and the tropical Atlantic after the closure of the Isthmus of Panama. The time-honored approach of comparing patterns found in many groups of organisms will determine their generality.

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